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The Characterization of IgG Receptor Induced by Human Cytomegalovirus[1] (39767)

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Introduction. Infection with herpes simplex virus (HSV) induces an IgG receptor on the surface of host cells (1), whereas infection with human cytomegalovirus (HCMV) induces the formation of IgG receptors in host-cell cytoplasm in the form of a cytoplasmic inclusion body (2, 3). Although these IgG receptors resulting from the infection of HSV and HCMV are morphologically distinguishable, both types are identifiable as Fc receptors by immunofluorescent techniques or by radioimmunoassay (4, 5). To understand the chemical nature and biological function of IgG receptor, IgG receptor from HCMV-infected cells was isolated and characterized by using immunoprecipitation techniques followed by polyacrylamide gel electrophoresis (PAGE).

Materials and methods. Virus and cell. The Towne strain of HCMV was used in these experiments for the infection of WI38 human diploid cells obtained from Dr. L. Hayflick, Stanford University. The history of the virus strain and the conditions of cell culture have been described previously (2). To infect the cultures, the multiplicity of infection of approximately 1 was used throughout the experiments.

Isotopes and antisera. L-[^{3}H]amino acid (^{3}H-AA), ^{14}C-labeled L amino acid (^{14}C-AA), and [^{14}C]glucosamine were purchased from New England Nuclear Corp., Boston, Mass. Anti-human immunoglobulin G (IgG), anti-rabbit immunoglobulin (Ig), and rabbit IgG were purchased from Hyland, Costa Mesa, Calif. Normal human sera were obtained from five children less than 3 years of age; these sera were HCMV-negative as determined by a complement-fixing (CF) test.

Condition of labeling. Cells were grown in Eagle's minimal essential medium (MEM) with 10% fetal calf serum, and the confluent culture of cells was infected with HCMV at a multiplicity of 1. Medium containing one-fifth the normal concentration of amino acids [1/5 basal minimal essential medium (BME)] was used for labeling cell proteins. ^{3}H-AA, ^{14}C-AA, and [^{14}C-]glucosamine were added to 5, 1, and 1 μCi/ml of 1/5 BME, respectively. After a 1-hr period for virus adsorption, cultures were maintained with isotope media until the time of harvest.

Preparation of proteins for indirect immunoprecipitation test. Cells were trypsinized and washed two times with Ca^{2+}- and Mg^{2+}-free phosphate-buffered saline (PBS) at a concentration of 2×10^6 cells/ml. Cells were sonicated at maximum output for 2 min with Sonifer Cell Disruptor (Heat System Co., Long Island, N. Y.) and then sedimented at 2000 rpm for 10 min. The proteins remaining in the supernatant were used in the indirect immunoprecipitation test and were stored at $-20°$ until used.

Indirect immunoprecipitation test. IgG was isolated from human sera by gel filtration through a Sephadex G-200 column (6). Briefly, crude γ-globulin was obtained from HCMV-negative human sera by two successive precipitations with a 1/3 concentration of saturated ammonium sulfate. For further purification of IgG, the precipitated γ-globulin was collected by centrifugation at 6000g for 20 min and passed through a Sephadex G-200 column (3×60 cm^2). IgG thus obtained was dissolved to 3 mg/ml. The amounts of proteins were measured by Lowry's method (7).

The indirect immunoprecipitation test followed the method of Horwitz and Scharff (8). Briefly, cell extracts were incubated with 0.15 mg/ml of IgG for 30 min at 37° with occasional shaking. Anti-IgG (1 mg/ml) was added and the IgG–protein complex was allowed to precipitate during an addi-

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tional 2-hr incubation. The precipitate was suspended in 0.2 ml of a 2-mercaptoethanol (1%), SDS (1%), and 0.5 *M* urea solution. After heating the sample to 100° for 2 min, dialysis was carried out against a 0.01 *M* phosphate buffer, 0.1% SDS, and 0.1% 2-mercaptoethanol for 16 hr.

Polyacrylamide gel electrophoresis. PAGE (7.5%) was performed following Maizel's method (9). The gels were made in a column of 0.6 × 15 cm and run for 16 hr at 6 mA/tube. After electrophoresis, the gel was sliced into 1-mm fractions and each slice was dissolved in 0.2 ml of 30% H_2O_2 by incubation overnight at 70° in a closed vial. Aquasol (NEN) was added to each sample. The samples were counted with a liquid scintillation counter.

Results. Indirect immunoprecipitation test against infected and uninfected cells. Previously cells infected with HCMV were reported to display fluorescent staining in their cytoplasm when examined with HCMV-CF-negative sera. Such cytoplasmic staining proved not to be specific to viral antigen of HCMV, but rather was a result of binding by the IgG receptor (2, 3, 4, 5).

To characterize further the IgG receptor, an indirect immunoprecipitation test utilizing purified HCMV-CF-negative human IgG or rabbit IgG was performed, comparing ^{3}H-AA-labeled extracts of HCMV-infected and noninfected cells. As shown in Fig. 1, the amounts of ^{3}H-AA (radioactivity) precipitated in receptor IgG-anti-IgG complexes were greater in HCMV-infected cells than in noninfected cells. In the same way, rabbit IgG precipitated infected cell protein to approximately the same amount that human IgG did.

Immunoprecipitation of Ig receptor during the course of infection. By the indirect immunofluorescence test, HCMV-infected cells were reported to have stained fluorescent with CF-negative sera at 24 hr postinfection (p.i.); at 48 hr p.i., immunofluorescent antigens were well-localized in the cytoplasm (2). To determine the time sequence of synthesis of IgG receptor during the normal course of infection, proteins synthesized at various stages of the infective process were tested by indirect immunoprecipitation test. Infected cells and noninfected cells of growing phase were labeled with 5 mCi of ^{3}H-AA for 24-hr intervals. The cell proteins were extracted and immunoprecipitated as described in *Materials and methods.* Table I shows that in infected cells, between 12.5 and 14% of the radioactivities were precipitated per 24 hr, while in noninfected cells, 2.8% were precipitated. The fraction of infected cell proteins precipitated was approximately constant until 72 hr p.i., indicating that the synthesis of IgG receptor was started from an early stage of the infective process and continued for 3 days.

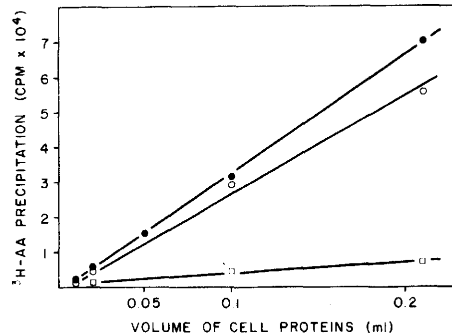

FIG. 1. Immunoprecipitation of IgG receptor. Cells were labeled with ^{3}H-AA (5 μCi/ml) continuously in 1/5 BME for 3 days. Cells were washed with PBS, sonicated, and centrifuged at 2000 rpm for 10 min to remove large debris. The same radioactivities (2.1 × 10^7 cpm/ml) were adjusted for both HCMV-infected and noninfected cells, in which the protein amounts were 6.6 and 6.0 mg/ml, respectively. These proteins were diluted at a concentration of 1 mg/ml with PBS and the different amounts of samples were mixed with 0.15 mg of human and rabbit IgG and incubated at 37° for 30 min. Protein-IgG complexes were immunoprecipitated with 1 and 0.7 mg/ml of anti-human and anti-rabbit IgG with continuous incubation for 2 hr. Precipitated proteins were washed with cold PBS three times and collected on the membranes, dried, and counted by scintillation counter. ●——●, ^{3}H-AA precipitated by human IgG in IgG-anti-IgG complexes from infected cell protein; ○——○, ^{3}H-AA precipitated by rabbit IgG in IgG-anti-IgG complexes from infected cell protein; □——□, ^{3}H-AA precipitated by human IgG in IgG-anti-IgG complexes from noninfected cell protein.

Electrophoresis of IgG receptor during the course of infection. The IgG receptor precipitated by the indirect immunoprecipitation test that was synthesized during the course of HCMV infection was electropho-

TABLE I. IMMUNOPRECIPITATION RATE OF THE TIME COURSE OF INFECTION.[a]

	A, Immunoprecipitated count (cpm)	B, Input count (cpm)	$A/B \times 100$ (%)
Non-infected cell	7.2×10^3	2.5×10^5	2.8
HCMV-infected cell 0 to 24-hr pulse	2.5×10^4	2.0×10^5	12.5
HCMV-infected cell 24 to 48-hr pulse	2.8×10^4	2.04×10^5	14.0
HCMV-infected cell 48 to 72-hr pulse	1.8×10^4	1.42×10^5	12.7

[a] Infected cells were labeled with ^{3}H-AA (5 μCi/ml) in 1/5 BME at 24-hr intervals. Cell proteins were extracted and precipitated with human IgG and anti-human IgG. Immunoprecipitation was performed with 1.0 mg of anti-human IgG to precipitate whole IgG receptor from infected cell protein. The reaction mixture was 0.15 mg of human IgG to 0.1 mg of cellular proteins.

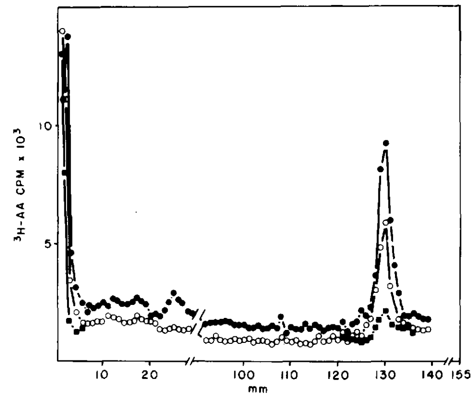

FIG. 2. PAGE profile of IgG receptors. Infected cells were labeled with ^{3}H-AA (5 μCi/ml) in 1/5 BME continuously and harvested at various times p.i. of HCMV. Cell proteins were extracted and precipitated with human IgG and anti-human IgG as described previously. Precipitated protein was dissolved in 0.2 ml of 1% SDS that contained 1 mM 2-mercaptoethanol; incubation followed in boiling water for 2 min. Fifty microliters of protein solution was applied on a PAGE column and run for 16 hr at 6 mA for each column. ●——●, 72 hr p.i.; ○——○, 48 hr p.i.; ■——■, 24 hr p.i.

resed to determine the size of polypeptide chain and the synthesis of IgG receptor. HCMV-infected cells were labeled with 5 μCi/ml of ^{3}H-AA continuously and harvested at 24, 48, and 72 hr p.i. Precipitated protein was solubilized and applied to the column of 7.5% polyacrylamide gel. Figure 2 shows a typical result of an electrophoretic separation. These three samples of IgG receptor migrated at the same position and showed a single peak in PAGE; the total ^{3}H counts of each peak were increased during the course of the infection. The results suggest that one type of polypeptide chain was clearly distinguishable and accumulated during the HCMV infection.

Electrophoresis of IgG receptor precipitated by human and rabbit IgG. To determine whether the two proteins precipitated with human and rabbit IgG are different, ^{3}H-labeled and ^{14}C-labeled infected cell proteins were precipitated with human and rabbit IgG, respectively. These samples were mixed and dissolved together and applied to a polyacrylamide gel column. Figure 3 shows that ^{3}H counts and ^{14}C counts migrated together and formed a single peak, suggesting that the IgG receptors precipitated with human and rabbit IgG are similar in molecular weight.

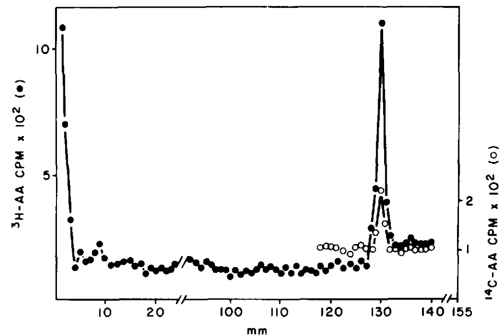

FIG. 3. PAGE profile of the IgG receptor precipitated with human IgG and rabbit IgG. Infected cells were labeled with ^{3}H-AA (5 μCi/ml) and ^{14}C-AA (1 μCi/ml) in 1/5 BME. ^{3}H-labeled infected cell proteins were precipitated with human IgG and ^{14}C-labeled proteins were precipitated with rabbit IgG. ^{3}H- and ^{14}C-labeled precipitated proteins were mixed, dissolved, and applied on PAGE column. ●——●, IgG receptor precipitated with human IgG; ○——○, IgG receptor precipitated with rabbit IgG.

Evidence of glycoprotein and molecular weight of IgG receptor. WI38 monolayer cells were infected with HCMV and labeled with [^{14}C]glucosamine and ^{3}H-AA. IgG receptor was precipitated with human IgG.

Figure 4 shows that ^{14}C and ^{3}H counts migrated together in the polyacrylamide gel, suggesting that the IgG receptor is a glycoprotein.

The molecular weight of the IgG receptor was determined by the method of Maizel (9), using proteins of known molecular weight as markers. ^{3}H-Labeled IgG receptor and marker proteins were mixed together and treated as previously described. Figure 5 shows that the IgG receptor migrated between bovine serum albumin and trypsin inhibitor; the molecular weight of the IgG receptor was 4.2×10^4 daltons.

Discussion. The data indicate that IgG receptor protein in HCMV-infected cells can be analyzed by indirect immunoprecipitation and PAGE. IgG receptor protein is apparently increased in infected cells following infection. This observation is in agreement with the data of Westmoreland and Watkins (1), who reported that infection with HSV induced an increase in IgG receptor.

It is unknown whether IgG receptor is a product of a viral or cellular gene, since receptors for the Fc fragment of IgG are present on both normal mouse macrophages (10) and B lymphocytes (11); the potentiality for production of the receptor may exist in the WI38 cell itself. It is possible that the insertion of virus into the cell activates the Fc receptor preexisting in a membrane (12).

In the case of HCMV infection, the IgG receptor is mainly localized in host cell cytoplasm, but some IgG receptor comes out on the plasma membrane of infected cells and develops the ability to bind both sheep red blood cells sensitized with rabbit anti-sheep red blood cell serum (2) and the human ^{125}I-labeled IgG (5). Although IgG receptor is mainly demonstrated in the cytoplasmic inclusion body of HCMV-infected cells by fluorescent staining, HCM-induced and HSV-induced IgG receptors are similar in terms of binding both sensitized sheep red cells and human IgG on cell membrane. However, there is no clear evidence that shows the distinct characters of the two IgG receptors. Since it has been postulated that IgG receptors on virus-infected cells may be occupied by immune-complex and block-cell-mediated cell destruction (13), further study should be directed to finding its significance in immunodefense mechanism.

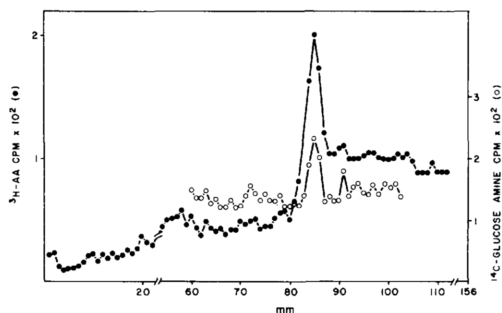

FIG. 4. PAGE profile of IgG receptor labeled with glucosamine-infected cells. Glucosamine-infected cells were double-labeled with ^{3}H-AA (5 μCi/ml) and [^{14}C]glucosamine (1 μCi/ml) in 1/5 BME. Extracted cell proteins were precipitated with human IgG. ●——●, ^{3}H counts; ○——○, ^{14}C counts.

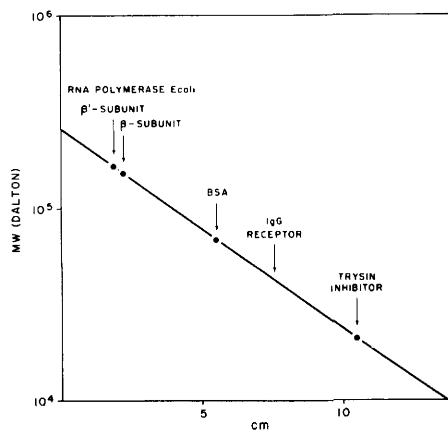

FIG. 5. Molecular weight of IgG receptor protein. ^{3}H-labeled IgG receptor protein was mixed with various indicator proteins, dissolved, and applied on a PAGE column. The positions of indicator proteins were measured by staining with coomassie blue, and the distance of IgG receptor protein was measured using 1 mm slices.

Summary. The IgG receptor was isolated from a lysate of human cytomegalovirus (HCMV)-infected human fibroblast cell (WI38) by the immunoprecipitation method. The IgG receptor migrated as a single peak in 7.5% of acrylamide gel electrophoresis and its molecular weight was 4.2×10^4 daltons. The IgG receptor was identified as glycoprotein and this protein was continuously synthesized at an almost constant rate from the early period of HCMV infection until 72 hr p.i.

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